Clin. exp. Immunol. (1992) **90**, 447–452

Fibroblast growth factors in connective tissue disease associated interstitial lung disease

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(Accepted for publication 11 August 1992)

SUMMARY

Fibrosis is a major cause of morbidity and mortality in chronic inflammatory diseases, especially interstitial pulmonary disorders. Fibroproliferation is an important part of this fibrotic response, and is mediated largely through growth factors such as platelet-derived growth factor (PDGF), insulin-like growth factor (IGF) I and tumour necrosis factor-alpha (TNF-α). Although there is some evidence implicating these cytokines in fibrotic disorders, strong evidence *in vivo* is almost non-existent. In order to ascertain the role that these factors play in inflammatory lung disorders associated with connective tissue diseases, alveolar mononuclear cells have been obtained from subjects by bronchoalveolar lavage and assessed for the spontaneous release of fibroblast growth factors. The study population consisted of subjects with a variety of different connective tissue disorders, both with and without inflammatory pulmonary complications. It was found that lavage cells spontaneously secreted fibroblast growth factor activity over 24 h with maximum activity detected at 6 to 12 h. Growth factor activity could be detected in most subjects with connective tissue disease-associated inflammatory lung disease and some normal subjects, but the amount of growth factor activity was much higher in the former than in the latter. By means of antibody depletion experiments all growth factor activity from lavage cells of normal patients was attributable to TNF-α while patients with interstitial lung disease secreted large amounts of PDGF and fibronectin in addition to TNF-α. Approximately 40–50% of the total released growth factor activity could be accounted for by PDGF, and 100% by the combination of PDGF, TNF-α and fibronectin. While TNF-α is released from the bronchoalveolar lavage cells of many subjects, in addition, many patients with interstitial lung disease also release spontaneously, large amounts of fibroblast growth factor activity attributable to PDGF and fibronectin.

Keywords interstitial lung disease connective tissue diseases scleroderma fibroblast growth factors alveolar macrophages TNF-α PDGF fibronectin

INTRODUCTION

Interstitial lung disease (ILD) is a relatively common complication of many connective tissue disorders, especially scleroderma [1,2], and is an important factor in determining disease morbidity and mortality. While there is some information on the pathogenesis of interstitial pulmonary disorders such as idiopathic pulmonary fibrosis and sarcoidosis, very little information is available in the case of connective tissue diseases associated ILD (CTD-ILD).

A major factor determining the long term outlook in patients with these disorders is the development of fibrosis, a process likely to be mediated, at least in part, by the local production of fibroblast growth factors. In order to assess whether alveolar mononuclear cells were spontaneously releasing factors capable of modulating fibroblast growth, and to determine the relative amounts of these factors released, cells were obtained from the lungs of patients with ILD or bronchiolitis associated with connective tissue diseases (CTD). These cells were obtained by bronchoalveoar lavage (BAL) and their spontaneously released mediators were collected by culture of these cells in serum-free conditions. The results of this study suggest that normal patients' cells obtained by BAL spontaneously release TNF-α while cells of patients with lung disease release TNF-α but also elevated amounts of PDGF and fibronectin, the combination of these three substances accounting for 100% of the total growth factor activity detected.

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MATERIALS AND METHODS

Patient selection

The study population consisted of a large group of patients with connective tissue diseases (both with and without respiratory

symptomatology) and a group of subjects without either pulmonary or systemic inflammatory disorders. It comprised a total of four 'normal' subjects with non-pulmonary malignancies, and 51 patients with connective tissue diseases: eight with Sjögren's syndrome alone; 10 with scleroderma alone, 20 with scleroderma plus Sjögren's syndrome; two with mixed connective tissue disease alone and two with mixed connective tissue disease plus Sjögren's syndrome; two with systemic lupus erythematosus alone and four with systemic lupus erythematosus plus Sjögren's syndrome; and three with a variety of other connective tissue diseases. Patients with scleroderma, systemic lupus erythematosus, rheumatoid arthritis or mixed connective tissue disease each fulfilled the American Rheumatism Association criteria for that diagnosis. For the purposes of this study, patients were considered to have Sjögren's syndrome when they gave a definite positive response to at least two out of three questions concerning the presence of dry eyes, dry mouth or recurrent salivary gland enlargement, and had additional objective confirmatory evidence for that diagnosis. The latter consisted of abnormalities of at least one of the following: Schirmer's test, salivary scan, minor salivary gland biopsy or antibodies to nuclear antigens SSA and/or SSB.

The study group and the method of their categorization has been previously described [3,4]. In brief, this involves the use of clustering techniques to define the multivariate structure inherent in the data, then discriminant analysis to group patients into distinct clusters and finally, an algorithm derived from the discriminant analysis has been developed that allows the assignment of any new patient into one of these clusters [3]. Discriminant analysis allowed grouping of all subjects into four distinct categories on the basis of respiratory function tests, gallium lung scans, and bronchoalveolar lavage cell counts and differentials [3]. In essence, from a pulmonary point of view, cluster 1 represents normal non-smokers, cluster 2 normal smokers, and cluster 3 patients with active ILD. Cluster 4 represents patients with a depressed maximal mid-expiratory flow rate (MMEFR), high lavage lymphocyte count and mildly elevated gallium index that probably have a bronchiolitis, although they may also have a mild lymphoid interstitial pneumonitis. For the purposes of this study, clusters 1 and 2 have been grouped together and defined as normals and clusters 3 and 4 grouped together and defined as having lung disease.

Respiratory evaluation

Respiratory physiology. A complete set of respiratory function parameters were obtained, including vital capacity (VC), alveolar volume (Va), maximal mid-expiratory flow rate (MMEFR), diffusion capacity (DLCO), and diffusion capacity corrected for the alveolar volume (KCO).

Gallium scan of the lung. Gallium scans were performed on each patient; 200–260 MBq of ^{67}gallium citrate was injected intravenously and scans were obtained 48 h later, using a posterior projection. Using published methods [5] and computerized data acquistion, a lung/liver gallium uptake index was calculated for the upper and lower regions of each lung, and then a mean value derived [5].

Bronchoalveolar lavage. This was carried out under local anaesthesia as previously described [6]. Briefly, it consisted of the passage, through the mouth, of a flexible fibreoptic bronchoscope which was wedged in turn into three subsegmental bronchi and into each, 100 ml of room temperature normal saline was instilled in four 25 ml aliquots. Each aliquot was aspirated *immediately* after instillation and the material was collected into a chilled, siliconized glass container and kept on ice till processed. Patients with chronic bronchitis or recent respiratory tract infections were excluded from the study. An aliquot of unprocessed lavage fluid was deposited onto a millipore filter [6]. This was washed, fixed and stained with haematoxylin and eosin and for non-specific esterase, in order to accurately differentiate macrophages from lymphocytes. Total cell counts were obtained using unprocessed lavage fluid and a counting chamber.

Collection and culture of bronchoalveolar cells

BAL fluid, collected in pre-cooled siliconized glass, was transferred to 50 ml polypropylene tubes (Crown Corning, NY) and centrifuged at 200 *g* at 4°C for 10 min. BAL fluid was decanted and the cells washed twice in Hank's balanced salt solution (HBSS) containing 50 U/ml penicillin G, 50 μg/ml streptomycin sulphate which were all purchased from Commonwealth Serum Laboratories (CSL; Victoria, Australia) and 2·5 mg/ml Fungizone (Flow Laboratories, McLean, VA). The total cell number and differential were estimated. Cell viability was ascertained by trypan blue (Flow Laboratories) exclusion. The freshly washed and isolated BAL cells were adjusted to a density of 1×10^6 cells/ml in *serum free* Medium 199 containing 2 mM glutamine, 20 mM HEPES, 50 U/ml penicillin G, 50 μg/ml streptomycin sulphate (all from CSL) and 2·5 mg/ml Fungizone and cultured for 24 h in 50 ml polypropylene tubes. At the appropriate time, the supernatants were collected by centrifugation, filtered with 0·2 micron filters (Gelman Sciences Inc., Michigan), aliquoted and stored at 70°C for analysis.

Bioassay for fibroblast growth factor activity

The supernatants were assayed according to the method of Thornton *et al.* [7]. Briefly, a human neonatal lung fibroblast cell line (CCD-34Lu) purchased from the ATCC (Rockville, MD) was grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% FCS, 20 mM HEPES, 2 mM glutamine and 5 μg/ml gentamycin (all from CSL). Cells were passaged at confluence using 0·5% trypsin-0·02% EDTA (Flow Laboratories) and split in a ratio of 1:5 in 75 cm^2 flasks. Only passages 3–10 were used for the assay. Fibroblasts were seeded in 100 μl aliquots in 96-well flat-bottomed tissue culture plates (Crown Corning) at a density of 1000 cells per well in medium containing a previously determined growth arresting concentration of FCS (0·2%). After 3 days incubation at 37°C, the medium was replaced with 200 μl aliquots of fresh medium containing 0·2% FCS, the supernatant (1/2 dilution) and 2 μCi/ml tritiated thymidine (20 Ci/mmol, Amersham, Australia). Each 96-well plate contained a positive control (10% FCS) and gave a response of at least 10 times the negative (background) control (0·2% FCS). The statistical difference between medium alone and test supernatants was ascertained using Student's *t*-test. Results were expressed as a percentage response index whereby Per cent response = [(d/min test supernatant − d/min background)/(d/min positive control − d/min background)] × 100. A patient was deemed to be growth factor positive if the culture supernatant, at a dilution of 1 in 25, was statistically significantly greater than the control.

Characterization of fibroblast growth factors

Characterization of fibroblast growth factors was determined by depletion studies using solid phase bound ligands as previously described [8]. In this instance antibodies were coupled to Affi-Gel 10 (Bio-Rad Laboratories Pty Ltd, Sydney, Australia) according to the manufacturer's instructions. Antibodies used were polyclonal rabbit anti-human IL-1α (Genzyme Corporation, Boston, MA), polyclonal rabbit anti-human IL-1β (Genzyme), polyclonal anti-human PDGF (Collaborative Research, Inc., Bedford, MA), monoclonal anti-TNF-α (Boehringer-Mannheim) and MoAb 3D1/2/1 against human insulin-like growth factor (IGF)-1 (a gift from Dr R. Baxter, Sydney). Heparin Sepharose CL-6B (Pharmacia LKB) because of its capacity to bind both acidic and basic fibroblast growth factors, PDGF and fibronectin were also used. Gelatin Sepharose CL-4B was used to bind any fibronectin.

Fifty μl Affi-Gel 10-antibody complex, Heparin Sepharose CL-6B and Gelatin Sepharose CL-4B (both purchased from Pharmacia LKB, Uppsala, Sweden) were washed three times in polypropylene Eppendorf tubes containing only HBSS to remove preservatives. A 1·5 ml supernatant generated from BAL cells was then added to the Affi-Gel 10-antibody complex, heparin sepharose or gelatin sepharose. The solution was mixed for 90 min by continuous inversion at room temperature followed by centrifugation at 5000 rev/min for 30 s. The supernatant was collected and filtered in 0·22 μm Millex-GV_4 filter units (Millipore, Bedford, MA). The samples were then assayed immediately for fibroblast growth factor activity.

In some instances, cell supernatants were also spun at 100 000 *g* for 1 h in an L8-70 M Ultracentrifuge (Beckman Instruments Inc., Palo Alto, CA) using a Ti70 rotor in order to remove any membrane fragments.

Detection of protease activity

Protease activity was determined by resorufin-labelled casein (Boehringer Mannheim) based on a previous method [9]. Synovial cell supernatants were incubated with resorufin-labelled casein at 37°C for 16 h and processed according to the manufacturer's instructions. The difference in absorbance at 574 nm of the sample solution against a blank at room temperature was determined.

RESULTS

Patient growth factor activity

Spontaneously released fibroblast growth factor activity (GFA) could be detected in about a third of normal subjects (clusters 1 and 2) and in about two-thirds of those with evidence of pulmonary pathology, whether ILD (cluster 3) or bronchiolitis (cluster 4) (Fig. 1). This difference was statistically significant using a χ^2 analysis ($P<0{\cdot}004$). No relationship between cell differentials and GFA was established.

Quantification of fibroblast growth factor activity

Quantification was undertaken in 14 subjects known to release fibroblast GFA. The titre of growth factor activity at 6 h for six normal patients was 1/125 while that for eight lung disease patients was greater than 1/625 (data not shown). Additionally, as expected the normals (mean = 1850 ± 120) had lower thymidine incorporation than the disease group (mean = 2931 ± 202) for the 1/5 titre. These results are statistically significant and $P=0{\cdot}0019$ using Student's *t*-test.

Spontaneous production of fibroblast growth factor activity with time

Patient samples spontaneously produced fibroblast growth factor activity when BAL cells were cultured in serum free conditions for 24 h. The time course of growth factor release from two normal patients and two lung disease patients at 6, 12, 18 and 24 h was determined. The production of growth factor activity was at a maximum between 6 and 12 h and a minimum at 24 h (data not shown). The titre of disease patients was greater than normal patients at each time interval (data not shown).

Since GFA decreased at 24 h for disease subjects, a number of experiments were carried out to attempt to identify the reasons. The amount of GFA was unchanged both at 6 and 24 h when the supernatants of normal and lung disease patients were assayed in the presence of indomethacin (data not shown). This suggests that there were probably no prostanoid-mediated inhibitory influences. A way of determining the presence of non-prostanoid inhibitors is to undertake mixing experiments, whereby varying ratios of a 6 h supernatant are mixed with the supernatant of a putative inhibitor (24 h). If an inhibitor is present, the GFA of the mixture will be substantially less than the average of the activities in the two supernatants. Mixing experiments using varying ratios of the 6 and 24 h supernatants showed no inhibition of GFA. This result argues against the presence of growth inhibitors (data not shown).

Protease activity at 24 h was determined to be on average 20–25 times greater (358 ± 86 ng trypsin) than at 6 h (16 ± 7 ng trypsin) suggesting that enzymic degradation may have been responsible for decrease in the GFA.

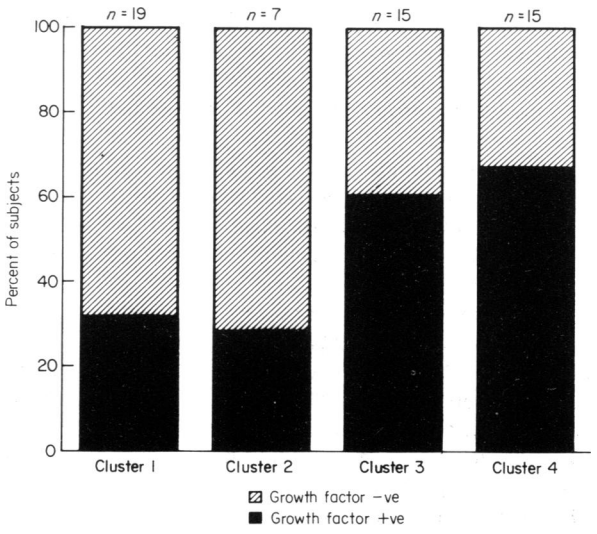

Fig. 1. Determination of spontaneous release of fibroblast GFA from BAL cells of 56 patients. Cluster 1 represents normal non-smokers, cluster 2 normal smokers, cluster 3 patients with interstitial lung disease and cluster 4 patients with bronchiolitis. The figure depicts the percentage of subjects exhibiting GFA in each category.

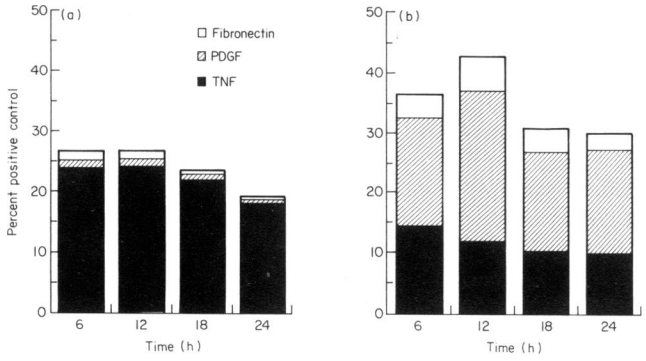

Fig. 2. Release of fibroblast GFA over time. Characterization of fibroblast growth factors was carried out by depletion using solid phase bound antibodies and gelatin Sepharose, in a normal patient (a) and a subject with disease patient (b). Fibroblast activity was determined by the uptake of tritiated thymidine (d/min). This diagram shows percentage of GFA due to the responsible growth factor. Results are expressed as percentage of the positive control. The bioassay was carried out three times using triplicate samples.

Characterization of fibroblast growth factors

Identification of fibroblast growth factors was undertaken by using solid phase bound antibodies in depletion experiments. Characterization of fibroblast growth factors was carried out in two ways. Firstly, the spontaneous production of growth factors of normal patients and lung disease patients was studied at 6, 12, 18 and 24 h. Secondly, stored patient samples containing growth factor activity, of six normal and eight lung disease patients collected at 6 h were studied.

Affi-Gel 10 or Sepharose alone had no significant effect on removal of growth factor activity nor did the anti-IL-1α, anti-IL-1β or anti-IGF-I (data not shown). In the time course study, both normal subjects had most of the growth factor activity removed by anti-TNF-α while only minimal amounts of the activity were removed by anti PDGF or Gelatin Sepharose which binds fibronectin (Fig. 2a). Heparin sepharose (which binds PDGF, fibronectin and the heparin binding fibroblast growth factors) removed no other activity other than that which was attributable to the PDGF and fibronectin (Fig. 2a). Figure 2 shows the proportion of growth factors ascribable to GFA. Patients with ILD produced TNF-α but in addition, also produced large amounts of PDGF and some fibronectin (Fig. 2b). There appeared to be a distinct peak of GFA at 12 h that was due largely to increased production of PDGF (Fig. 2b). Anti-PDGF, anti-TNFα and gelatin sepharose removed all the fibroblast growth factor activity (Fig. 2b).

The cell supernatants from 14 subjects exhibiting significant GFA (six cluster 1 or 2 subjects (Fig. 3a) and eight cluster 3 or 4 subjects (Fig. 3b)) were randomly selected and analysed in antibody depletion studies. The normal (cluster 1+2) subjects all had substantially less growth factor activity than the lung disease (cluster 3+4) subjects. In addition, in five of the six cluster 1+2 subjects, the GFA was due almost exclusively to TNF-α, whilst in the cluster 3+4 subjects, there was a large amount of activity attributable to PDGF and to a lesser extent fibronectin (Fig. 3b).

Membrane associated growth factor activity

After ultracentrifugation of cell supernatants, a decrease of 10–

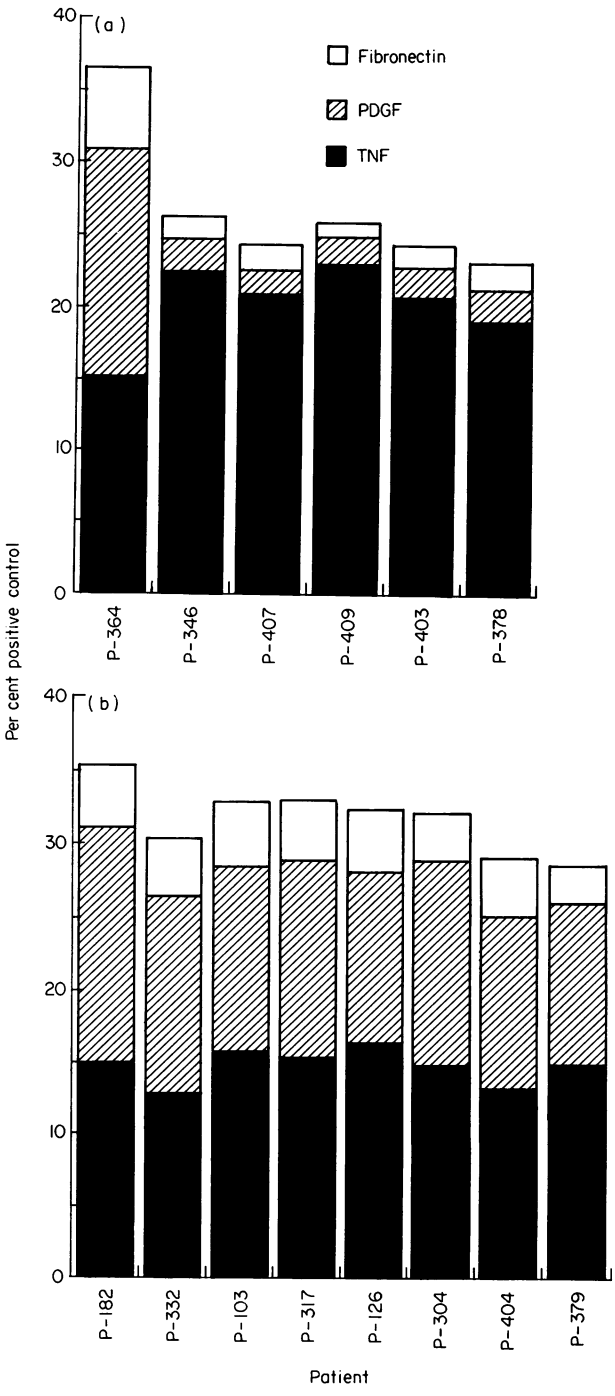

Fig. 3. Characterization of fibroblast growth factors in (a) six normal subjects and (b) eight subjects from the disease group. Fibroblast GFA was determined by the uptake of tritiated thymidine (d/min) and the data are shown as the percentage of GFA due to the reponsible growth factor, with results expressed as percentage of the positive control. The bioassay was carried out three times using triplicate samples.

20% occurred in GFA of disease as well as in normal patient groups (Table 1). Resuspension of the pellets and antibody depletion studies revealed this activity in the pellet to be due entirely to TNF-α (data not shown). This suggests that some TNF-α activity may be due to artefactual membrane fragmentation.

Table 1. Ultracentrifugation of BAL cell supernatants

Source of material	Growth factor activity (d/min)	
	Pre-ultracentrifugation	Post-ultracentrifugation
Control medium		
0·20% FCS	569±89	601±102
10% FCS	9306±1198	9876±791
Conditioned medium from normal subjects		
P-346	2530±139	2118±154
P-407	2220±219	1853±192
P-409	2487±302	2002±286
Conditioned medium from disease subjects		
P-182	3670±432	3178±342
P-103	3291±101	2853±333
P-317	3381±291	2953±171

DISCUSSION

Interstitial lung diseases are a group of chronic inflammatory disorders of the lower respiratory tract which are characterized by increased numbers of activated alveolar macrophages and increased numbers of fibroblasts within the alveolar wall [10] and a variable amount of fibrosis. Alveolar macrophages have been shown to produce mediators which regulate various aspects of the inflammatory response and fibroproliferation. These substances include TNF-α [11–13], IL-1 [11,14–16], alveolar macrophage-derived growth factor (AMDGF) [10,15], fibronectin [15,17], insulin-like growth factor I (IGF-I) [18], colony stimulating factor [11], prostaglandin E_2 (PGE_2) [15,19], transforming growth factor-β (TGF-β) [20] and PDGF [21,22].

While fibroblast growth factors of various types have been detected in a number of animal models, studies looking at spontaneous release of these factors in human diseases are very limited. There have been no studies in ILD-CTD, and in none of the studies has an attempt been made to identify all the GFA producing substances and define their relative biological importance. This study has demonstrated that BAL cells from both normal subjects and patients with lung disease are capable of spontaneously releasing fibroblast GFA (Fig. 1). However, the composition and amount of fibroblast GFA appear quite different when comparing the normal and ILD patients (Fig. 2). A representative time course of one of the normal patients, shows that most of the growth factor activity is due to TNF-α with small amounts of PDGF and fibronectin released over 24 h. In the disease group, however, more than half of the GFA is due to PDGF (Fig. 2), suggesting its disease specific synthesis. As depletion studies removed essentially all growth factor activity, the presence of any other secreted fibroblast growth factors is extremely unlikely.

In the 14 samples assessed in detail, there appeared to be good separation in the amount and type of GFA detected in normals and those with lung disease. That is, release of TNF-α by both groups, but release of PDGF essentially only by the disease group (Figs. 3a and 3b). The result of only one subject was other than expected and it is possible that this represents a misclassification of that patient (Fig. 3a). Alternatively this may represent a CTD subject in the very early phases of inflammatory lung disease. There was no clear difference in the growth factor activity within the two groups comprising the lung disease subjects, i.e. cluster 3 subjects with ILD and cluster 4 subjects with bronchiolitis. Lung disease subjects with either ILD or bronchiolitis produced both PDGF and TNF-α, suggesting that the same mediators that play a major part in the interstitial fibrosis seen in ILD, play and important role in the peribronchial fibrosis in bronchiolitis.

CONCLUSION

Alveolar mononuclear cells from both normal and lung disease patients obtained by bronchoalveolar lavage spontaneously released fibroblast growth factors for 24 h. Growth factor activity was a maximum at 6–12 h and tended to be present in smaller amounts in the normals than the ILD subjects. However, by means of antibody depletion experiments normal patients were found to secrete TNF-α while patients with ILD secreted not only TNF-α but also large amounts of PDGF and fibronectin. The dominant fibroblast growth factors spontaneously released in the ILD appear to be PDGF, TNF-α and a smaller amount of fibronectin, and these together account for all detectable fibroblast growth factor bioactivity.

ACKNOWLEDGMENTS

This work was supported in part by grants from the National Health and Medical Research Council of Australia and the Arthritis Foundation of Australia. This work could not have been carried out without the generous assistance of patients with connective tissue diseases especially those of the Scleroderma and Lupus Association of NSW.

REFERENCES

1 LeRoy EC, Black C, Fleischmajer R, Jablonska S, Krieg T, Medsger TA Jr, Rowell N, Wolheim F. Scleroderma (systemic sclerosis): classification, subsets and pathogenesis. J Rheumatol 1988; **15**:202–5.
2 LeRoy EC. Systemic sclerosis (scleroderma). In: Wyngaarden JB, Smith LH. eds. Cecil Textbook of Medicine. 18th edn. Philadelphia: W.B. Saunders, 1988.
3 Cairns D, Shelley L, Burke WM, Bryant DH, Yeates M, Morgan GW, Penny R, Breit SN. Interstitial lung disease associated with connective tissue diseases. The use of statistical structure analysis in model development. Am Rev Respir Dis 1991; **143**:1235–40.
4 Cairns D, Shelley L, Burke W, Bryant DH, Yeates M, Penny R, Breit SN. The differing patterns of interstitial lung involvement in connective tissue diseases. J Rheumatol in press.
5 Bisson G, Drapeau G, Lamoureux G, Cantin A, Rola-Pleszcynski M, Begin R. Computer-based quantitative analysis of gallium-67 uptake in normal and diseased lungs. Chest 1983; **84**:513–17.
6 Saltini C, Hance AJ, Ferrans VJ, Basset F, Bitterman, PB, Crystal RG. Accurate quantification of cells recovered by bronchoalveolar lavage. Am Rev Resp Dis 1984; **130**:650–8.
7 Thornton SC, Por SB, Walsh BJ, Penny R, Breit SN. Interaction of immune and connective tissue cells: 1. The effect of lymphokines and monokines on fibroblast growth. J Leukocyte Biol 1990; **47**:312–20.
8 Thornton SC, Por SB, Penny R, Richter M, Shelley L, Breit SN. Identification of the major fibroblast growth factors spontaneously released in inflammatory arthritis as PDGF and TNF alpha. Clin Exp Immunol 1991; **86**:79–86.
9 Twining SS. Fluorescein isothiocyanate-labeled casein assay for proteolytic enzymes. Anal Biochem 1984; **143**:30–4.
10 Bitterman PB, Adelberg S, Crystal RG. Mechanisms of pulmonary fibrosis. Spontaneous production of the alveolar macrophage-

derived growth factor in the interstitial lung disorders. J Clin Invest 1983; **72**:1801–13.

11 Becker S, Devlin RB, Haskill JS. Differential production of tumor necrosis factor, macrophage colony stimulating factor, and interleukin 1 by human alveolar macrophages. J Leukocyte Biol 1989; **45**:353–61.

12 Piguet PF, Collart MA, Grau GE, Sappino AP, Vassalli P. Requirement of tumor necrosis factor for development of silica-induced pulmonary fibrosis. Nature 1990; **344**:245–7.

13 Bachwich PR, Lynch JP, Larrick J, Spengler M, Kunkel SL. Tumor necrosis factor production by human sarcoid alveolar macrophages. Am J Pathol 1986; **125**:421–5.

14 Wewers MD, Herzyk DJ. Alveolar macrophages differ from blood monocytes in human IL-1β release. Quantitation by enzyme-linked immunoassay. J Immunol 1989; **143**:1635–41

15 Bitterman PB, Wewers MD, Rennard SI, Adelberg S, Crystal RG. Modulation of alveolar macrophage-driven fibroblast proliferation by alternative macrophage mediators. J Clin Invest 1986; **77**:700–8.

16 Wewers MD, Rennard SI, Hance AJ, Bitterman PB, Crystal RG. Normal human alveolar macrophages obtained by bronchoalveolar lavage have a limited capacity to release interleukin-1. J Clin Invest 1984; **74**:2208–18.

17 Ozaki T, Moriguchi H, Nakamura Y, Kamei T, Yasuoka S, Ogura T. Regulatory effect of prostaglandin E_2 on fibronectin release from human alveolar macrophages. Am Rev Respir Dis 1990; **141**:965–9.

18 Rom WN, Basset P, Fells GA, Nukiwa T, Trapnell B, Crystal RG. Alveolar macrophages release an insulin-like growth factor I-type molecule. J Clin Invest 1988; **82**:1685–93.

19 Monick M, Glazier J, Hunninghake GW. Human alveolar macrophages suppress interleukin-1 (IL-1) activity via the secretion of prostaglandin E_2. Am Rev Respir Dis 1987; **135**:72–7.

20 Assoian RK, Fleurdelys BE, Stevenson HC, Miller PJ, Madtes DK, Raines EW, Ross R, Sporn MB. Expression and secretion of type β transforming growth factor by activated human macrophages. Proc Natl Acad Sci USA 1987; **84**:6020–24.

21 Mornex JF, Martinet Y, Yamauchi K, Bitterman PB, Grotendorst GR, Chytil-Weir A, Martin GR, Crystal RG. Spontaneous expression of the c-*sis* gene and release of a platelet-derived growth factorlike molecule by human alveolar macrophages. J Clin Invest 1985; **78**:61–6.

22 Martinet Y, Rom WN, Grotendorst GR, Martin GR, Crystal RG. Exaggerated spontaneous release of a platelet-derived growth factor by alveolar macrophages from patients with idiopathic pulmonary fibrosis. N Eng J Med 1987; **317**:202–9.